

A well-controlled BiID design for endogenous bait proteins

Supplementary Information

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PCR	Construct/Purpose	Forward primer (5'-3')	Reverse primer (5'-3')
TP53 5'HR	pAav-p53-T2A-BirA*	CACTAGGGTCTCGGGCCGAAACGCG TCAGCCCTGCACAGACATTT	CGACGAAGCTTGGGCCAGGATTCTCTCG ACGTCAACCGCATTTAGCAGACTTCTCTG CCCTCTCACTGCCCATATGGTCTGAGTC GGCCCTTCTG
TP53 3'HR	pAav-p53-T2A-BirA*	GAAGTTATGGTACCCATTCTCCACTTCTGT TCC	GGGTTCTGCGGCCGTTTGTAGCAGAT CACGCCACTCCACTCC
BirA*	pAav-p53-T2A-BirA*	CATCCACGGCTCATATGGCAGTGGAGAG GGCAGAGGAAGTCTGCTAACCTCGGGGA CGTCGAGGAGAATCTGGCCCAAAGGACA ACACGGTCCCCCTGAAGCTGATCGC	GCTAGCAAGCTTCATTACTCTCGCGCTT CTCAGGG
Lox-Neo-Lox	pAav-p53-T2A-BirA*	CAGAGAAGTAATGAAAGCTTGCTAGCAT ACTTCGTATAGCATACATTATAACGAAGTT TC	GGAACAAGAAGTGGAGAATGGGTACCAT ACTTCGTATAATGTATGCTATACGAAGTTA TAGCTGGTCT
TP53 junction 5'HR	Screening	ATCAGCCAAGATTGCACCAT	TCAGCTCTCCGATTCTGTCC
TP53 junction 3'HR	Screening	GGGAGGGATTGGGAAGACAAT	CCAGTCTCCAGCCTTGTTC
Cassette deletion	Cre-Lox recombination	ACTCATGTTCAAGACAGAAGGGCCTGAC	TGACGCACACCTATTGCAAGCAAGG
WT allele	Sanger sequencing	ACATATTGCAATGGGTGTG	CCTAGAATGTGGCTATTGTAAC
Knock-in allele	Sanger sequencing	CACTAGGGTCTCGGGCCGAAACGCG TCAGCCCTGCACAGACATTT	ATTACTCTCTCGCGCTCTCAG
T2A region	Illumina sequencing	TCGTCGGCAGCGTCAGATGTGATAAGAG ACAGGGGTAGTCACCTCCGCC	GTCTCGTGGCTGGAGATGTATAAGA GACAGCTGCCGTTGGCCAGCAGGG
Neomycin	SB probe	TGCTCCTGCCGAGAAAGTAT	GCGATGCAATTCTCTCATT
T2A	pMet7 mutagenesis	CCTTTGGGCCAAAATTCTCTCGACGTCCC CGCA	TGCGGGGACGTCGAGGAGAATTGGCCC AAAGG

Table S1. Primers used for cloning or screening. HR: homology region; SB: Southern blot. The T2A sequence was encoded in the reverse p53 5'HR and forward BirA* primers.

	Sequence
gRNA1 cassette	TGTACAAAAAAGCAGGCTTAAAGGAACCAATTCACTCGACTGGATCCGGTACCAAGGTGGCAGGAAGAGGGCCTATTCAT <i>GATTCC</i> TCATATTGCATATAACGATAACAAGGCTTAGAGAGATAATTAGAATTAAATTGACTGTAAACACAAAGATATTAGTACAA AATACGTGACGTAGAAAAGTAATAATTCTGGTAGTTGCAGTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAAC TTGAAAGTATTGATTTCTGGCTTATATATCTTGAAAGGACGAAACACCG ATTCTCCTCGACGTCCCCGC <u>GTTTAGAGCTAG</u> <u>AAATAGCAAGTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGT</u> TTCTAGACCCAGCTTCTG TACAAAGTTGGCATT
gRNA2 cassette	TGTACAAAAAAGCAGGCTTAAAGGAACCAATTCACTCGACTGGATCCGGTACCAAGGTGGCAGGAAGAGGGCCTATTCAT <i>GATTCC</i> TCATATTGCATATAACGATAACAAGGCTTAGAGAGATAATTAGAATTAAATTGACTGTAAACACAAAGATATTAGTACAA AATACGTGACGTAGAAAAGTAATAATTCTGGTAGTTGCAGTTAAAATTATGTTAAAATGGACTATCATATGCTTACCGTAAC TTGAAAGTATTGATTTCTGGCTTATATATCTTGAAAGGACGAAACACCG TCTGGCCAAAGGACAACAGTTTAGAGCTA <u>GAAATAGCAAGTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGT</u> TTCTAGACCCAGCTTCTG GTACAAAGTTGGCATT

Table S2. gRNA gBlock sequences cloned in pCR-Blunt® vector for transfection. Italics: U6 promoter sequence. Bold: gRNA sequence. Underlined: universal gRNA scaffold. Sequences were ordered as IDT gBlocks and blunt-end ligated in pCR-Blunt® vectors prior to transfection in target cells together with BE3. After parallel testing it was decided to use gRNA2 for endogenous mutagenesis.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Nucleoplasm	441	1.89E-116	2.95	1.28E-113
Cell-cell adherens junction	118	2.91E-66	6.81	1.97E-63
Cytosol	397	1.05E-63	2.23	7.14E-61
Cytoplasm	500	3.16E-52	1.78	2.15E-49
Catalytic step 2 spliceosome	52	1.24E-40	10.53	8.41E-38
Nucleus	473	1.54E-36	1.63	1.05E-33
Nucleolus	143	6.30E-35	3.11	4.28E-32
Membrane	252	1.21E-33	2.13	8.20E-31
Spliceosomal complex	47	1.61E-33	9.32	1.09E-30
Proteasome complex	29	3.38E-20	9.01	2.30E-17
Nuclear speck	49	6.67E-19	4.54	4.53E-16
Focal adhesion	69	3.30E-18	3.29	2.24E-15
Microtubule	58	1.62E-16	3.48	7.54E-14
Proteasome accessory complex	14	5.45E-14	15.35	3.70E-11
Eukaryotic 43S preinitiation complex	13	2.07E-13	16.15	1.41E-10
Protein complex	60	4.64E-12	2.71	3.15E-09
Eukaryotic 48S preinitiation complex	12	1.11E-11	14.91	7.57E-09
Intracellular ribonucleoprotein complex	31	2.48E-11	4.25	1.69E-08
Actin cytoskeleton	40	2.67E-11	3.42	1.81E-08
Extracellular exosome	228	2.95E-11	1.51	2.01E-08

GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Poly(A) RNA binding	295	1.98E-123	4.60	1.49E-120
Protein binding	782	1.79E-85	1.57	1.35E-82
Cadherin binding involved in cell-cell adhesion	116	1.26E-67	7.04	9.45E-65
RNA binding	115	2.81E-35	3.70	2.11E-32
Unfolded protein binding	36	1.21E-17	5.76	9.12E-15
Nucleotide binding	59	1.03E-13	2.98	7.77E-11
Transcription coactivator activity	47	8.06E-13	3.34	6.06E-10
Translation initiation factor activity	23	8.12E-13	6.64	6.11E-10
Histone acetyltransferase activity	19	4.61E-11	6.97	3.47E-08
Chromatin binding	57	1.38E-10	2.57	1.03E-07
Threonine-type endopeptidase activity	13	1.91E-10	10.90	1.43E-07
ATP binding	144	2.00E-10	1.70	1.50E-07
RNA polymerase II distal enhancer sequence-specific DNA binding	20	1.82E-09	5.42	1.37E-06
Structural constituent of cytoskeleton	24	4.35E-08	3.84	3.27E-05
RNA polymerase II core binding	10	9.30E-07	8.38	6.99E-04
Ubiquitin protein ligase binding	39	9.74E-07	2.39	7.32E-04
Nucleosomal DNA binding	14	1.03E-06	5.36	7.75E-04
Actin filament binding	24	1.35E-06	3.20	0.0010
ATPase activity	29	1.54E-06	2.79	0.0012
mRNA binding	23	1.92E-06	3.24	0.0014

Table S4: GO analysis of the results from an analysis of a transient transfection p53 BiOID experiment using a mock vector as control. This table shows the top 20 enriched ‘cellular component’ (top panel) or ‘molecular function’ terms (bottom panel). Count: number of proteins identified related to a certain GO term. Bonferroni: Bonferroni-corrected p-values.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Cytosol	194	4.17E-44	2.67	1.72E-41
Extracellular exosome	166	1.40E-36	2.69	5.81E-34
Focal adhesion	51	2.87E-24	5.94	1.19E-21
Membrane	120	5.63E-22	2.49	2.33E-19
Cytoplasm	205	7.38E-22	1.79	3.06E-19
Myelin sheath	31	2.21E-20	9.29	9.16E-18
Cell-cell adherens junction	40	3.03E-18	5.64	1.26E-15
Extracellular matrix	36	4.44E-16	5.54	1.84E-13
Nucleoplasm	122	1.28E-14	2.00	5.29E-12
Mitochondrion	73	5.99E-13	2.50	2.48E-10
Ribosome	22	9.96E-11	6.04	4.12E-08
Endoplasmic reticulum chaperone complex	8	7.00E-10	33.13	2.90E-07
Melanosome	16	5.25E-09	7.22	2.18E-06
Cytosolic small ribosomal subunit	12	5.27E-09	11.39	2.18E-06
Proteasome complex	13	5.58E-09	9.87	2.31E-06
Nucleus	173	6.75E-09	1.46	2.80E-06
Mitochondrial matrix	27	1.62E-08	3.76	6.71E-06
Mitochondrial inner membrane	30	1.53E-07	3.10	6.34E-05
Proteasome core complex	8	2.04E-07	17.36	8.45E-05
Actin cytoskeleton	20	3.52E-07	4.18	1.46E-04

GO Molecular Function

Term	Count	P-value	Fold Enrichment	Bonferroni
Protein binding	323	1.13E-35	1.57	6.68E-33
Unfolded protein binding	35	4.30E-29	13.60	2.55E-26
Poly(A) RNA binding	83	7.20E-21	3.14	4.26E-18
Cadherin binding involved in cell-cell adhesion	40	5.65E-19	5.89	3.34E-16
ATP binding	83	1.42E-13	2.37	8.43E-11
Chaperone binding	18	4.22E-12	9.50	2.50E-09
Structural constituent of ribosome	26	8.05E-11	5.01	4.77E-08
Hsp70 protein binding	11	2.45E-09	14.25	1.45E-06
Heat shock protein binding	11	3.24E-08	11.19	1.92E-05
Threonine-type endopeptidase activity	8	3.14E-07	16.28	1.86E-04
GTP binding	28	3.79E-07	3.12	2.24E-04
GTPase activity	21	6.36E-07	3.84	3.76E-04
Protein kinase binding	27	8.56E-07	3.07	5.07E-04
Ubiquitin protein ligase binding	23	1.12E-06	3.42	6.63E-04
Biotin carboxylase activity	5	1.43E-06	42.74	8.49E-04
Adenyl-nucleotide exchange factor activity	6	1.55E-06	25.64	9.14E-04
Hsp90 protein binding	7	3.04E-05	11.08	0.018
Enzyme binding	22	3.88E-05	2.82	0.023
Structural constituent of cytoskeleton	12	5.25E-05	4.66	0.031
Transcription coactivator activity	18	7.67E-05	3.10	0.044

Table S5. GO analysis of the results from an analysis of a transient transfection p53 BiOID experiment using a bi-cistronic p53-T2A-BirA* expression construct as control. This table shows the top 20 enriched ‘cellular component’ (top panel) or ‘molecular function’ terms (bottom panel). Count: number of proteins identified related to a certain cellular component. Bonferroni: Bonferroni-corrected p-values.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Nucleoplasm	138	6.60E-75	4.99	1.41E-72
Mediator complex	21	7.50E-32	60.41	1.60E-29
Nucleus	130	1.96E-31	2.42	4.17E-29
Transcription factor TFTC complex	11	6.62E-18	79.11	1.41E-15
Nuclear chromatin	23	2.22E-17	12.00	4.74E-15
STAGA complex	10	1.40E-15	71.92	3.07E-13
Transcription factor TFIID complex	12	3.11E-14	33.56	6.62E-12
Nucleolus	37	1.18E-13	4.35	2.51E-11
NuA4 histone acetyltransferase complex	9	3.12E-12	50.34	6.64E-10
NuRD complex	9	3.12E-12	50.34	6.64E-10
SWI/SNF complex	8	4.91E-11	53.70	1.05E-08
Core mediator complex	6	5.29E-10	100.69	1.13E-07
NpBAF complex	7	7.51E-10	58.73	1.60E-07
MLL1 complex	8	1.06E-08	27.78	2.26E-06
Sin3 complex	6	1.07E-07	46.47	2.29E-05
Ubiquitin ligase complex	11	1.15E-07	10.25	2.46E-05
Protein complex	19	1.47E-07	4.64	3.13E-05
nBAF complex	6	1.66E-07	43.15	3.53E-05
Ada2/Gcn5/Ada3 transcription activator complex	6	3.56E-07	37.76	7.57E-05
Chromosome	10	9.75E-07	9.59	2.08E-04

GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Transcription coactivator activity	37	1.42E-30	13.84	3.43E-28
Protein binding	163	1.36E-27	1.72	3.27E-25
RNA polymerase II transcription cofactor activity	18	1.08E-24	46.38	2.61E-22
Histone acetyltransferase activity	18	4.76E-22	34.78	1.15E-19
Chromatin binding	34	1.89E-20	8.07	4.55E-18
Nucleosomal DNA binding	14	1.17E-15	28.23	2.94E-13
RNA polymerase II distal enhancer sequence-specific DNA binding	15	6.07E-15	21.40	1.47E-12
Vitamin D receptor binding	10	7.22E-15	61.84	1.74E-12
DNA binding	55	5.02E-14	3.05	1.21E-11
Histone deacetylase activity	12	2.05E-13	28.54	4.94E-11
Thyroid hormone receptor binding	10	6.09E-12	34.35	1.47E-09
Transcription cofactor activity	13	1.18E-11	16.98	2.85E-09
p53 binding	12	1.21E-10	16.61	2.92E-08
RNA polymerase II core promoter proximal region sequence-specific DNA binding	19	5.29E-08	4.96	1.27E-05
Ligand-dependent nuclear receptor transcription coactivator activity	9	6.43E-08	16.37	1.55E-05
Transcription factor binding	17	7.19E-08	5.55	1.73E-05
RNA polymerase II repressing transcription factor binding	7	3.43E-07	24.05	8.27E-05
Poly(A) RNA binding	33	3.76E-07	2.71	9.07E-05
Ubiquitin protein ligase activity	13	8.37E-07	6.45	2.02E-04
Lysine-acetylated histone binding	6	1.03E-06	30.92	2.47E-04

Table S7: GO analysis of significantly enriched proteins from converted p53-MUTT2A-BirA* HCT116 cells when compared to isogenic non-converted p53-T2A-BirA* cells. This table shows the top 20 enriched ‘cellular component’ (top panel) or ‘molecular function’ terms (bottom panel). Count: number of proteins identified related to a certain GO term. Bonferroni: Bonferroni-corrected p-values.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Nucleoplasm	234	3.17E-93	3.95	1.19E-90
Nucleus	250	5.05E-46	2.17	1.90E-43
Nucleolus	87	9.57E-35	4.77	3.61E-32
Catalytic step 2 spliceosome	32	3.66E-29	16.34	1.38E-26
Mediator complex	22	7.51E-27	29.52	2.83E-24
Cytosolic large ribosomal subunit	27	2.59E-26	18.65	9.77E-24
Nuclear speck	35	4.07E-21	8.18	1.54E-18
Ribosome	32	1.15E-20	9.05	4.34E-18
Nuclear chromatin	31	1.05E-17	7.54	3.97E-15
Cell-cell adherens junction	38	4.82E-17	5.53	1.82E-14
Spliceosomal complex	21	6.81E-15	10.49	2.55E-12
Membrane	103	7.48E-15	2.20	2.80E-12
STAGA complex	11	1.54E-14	36.90	5.82E-12
Transcription factor TFTC complex	11	1.54E-14	36.90	5.82E-12
Cytoplasm	180	9.19E-14	1.62	3.47E-11
NuA4 histone acetyltransferase complex	11	6.25E-13	28.70	2.36E-10
Focal adhesion	35	3.44E-12	4.20	1.30E-09
Intracellular ribonucleoprotein complex	21	9.97E-12	7.25	3.76E-09
Cytosol	124	5.12E-11	1.76	1.93E-08
Transcription factor TFIID complex	12	1.28E-10	15.66	4.84E-08

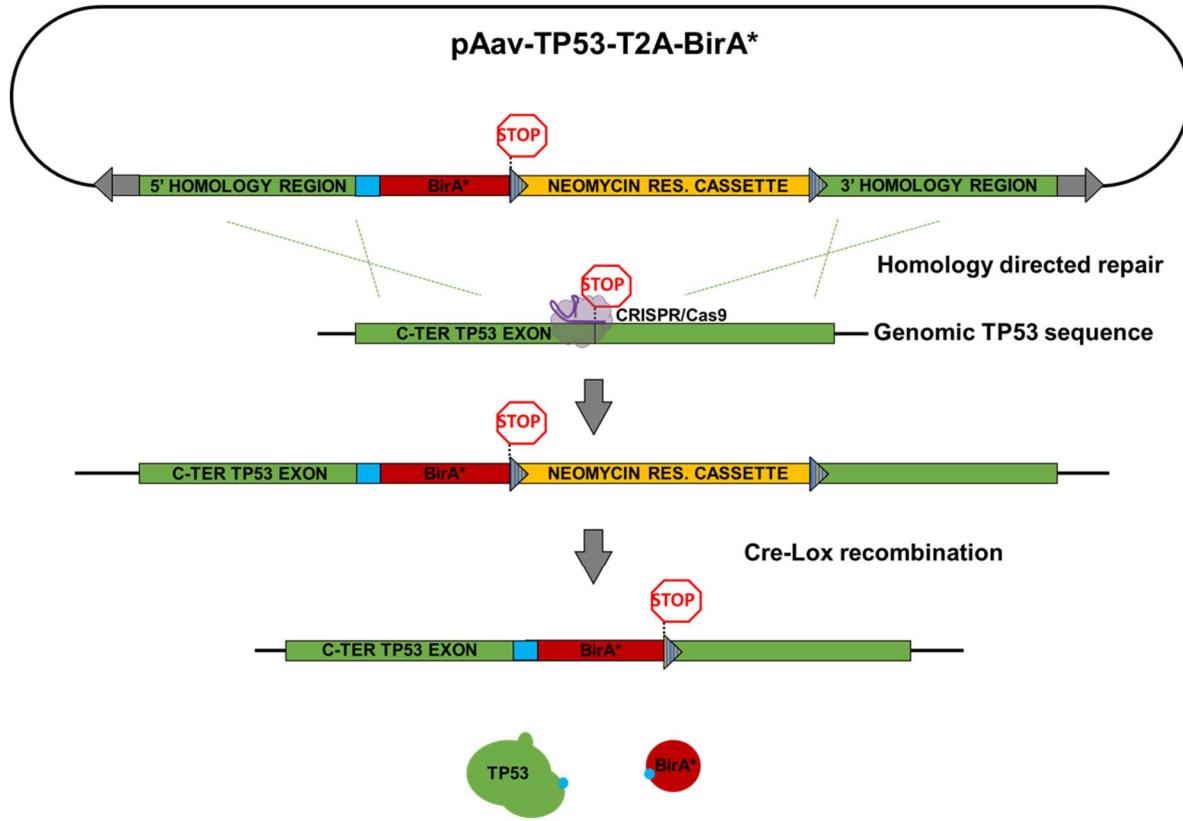
GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Poly(A) RNA binding	142	2.02E-67	5.44	8.46E-65
Protein binding	334	1.43E-45	1.65	6.00E-43
Transcription coactivator activity	44	1.61E-25	7.68	6.75E-23
RNA polymerase II transcription cofactor activity	19	1.38E-20	22.84	5.80E-18
Histone acetyltransferase activity	19	8.52E-18	17.13	3.58E-15
Structural constituent of ribosome	34	1.03E-17	6.63	4.32E-15
Cadherin binding involved in cell-cell adhesion	38	1.79E-17	5.67	7.53E-15
RNA binding	51	4.83E-17	4.04	2.03E-14
Vitamin D receptor binding	12	1.06E-15	34.63	4.66E-13
Nucleosomal DNA binding	16	5.60E-14	15.06	2.35E-11
Chromatin binding	39	6.21E-14	4.32	2.61E-11
RNA polymerase II distal enhancer sequence-specific DNA binding	17	1.08E-12	11.32	4.52E-10
DNA binding	85	4.01E-12	2.20	1.68E-09
Thyroid hormone receptor binding	12	7.92E-12	19.24	3.33E-09
Transcription cofactor activity	15	6.89E-10	9.14	2.89E-07
Histone deacetylase activity	12	7.94E-10	13.32	3.33E-07
P53 binding	13	3.62E-08	8.40	1.52E-05
Transcription factor binding	24	1.95E-07	3.66	8.18E-05
RNA polymerase II core promoter proximal region sequence-specific DNA binding	26	7.97E-07	3.17	3.35E-04
mRNA binding	15	1.14E-06	5.19	4.77E-04

Table S8: GO analysis of significantly enriched proteins from converted p53-MUTT2A-BirA* HCT116 cells

when compared to parental HCT116 cells. This table shows the top 20 enriched ‘cellular component’ (top panel) or ‘molecular function’ terms (bottom panel). Count: number of proteins identified related to a certain GO term. Bonferroni: Bonferroni-corrected p-values.

A.



B.

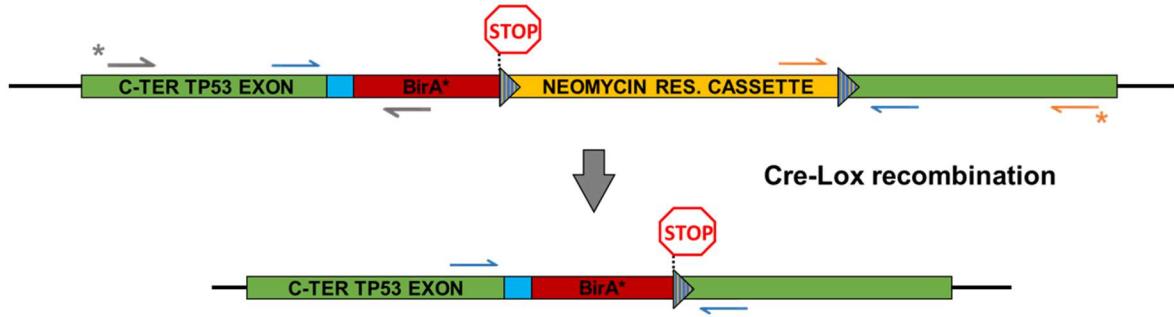


Figure S1. Targeting strategy and primer scheme used for PCR screening. (A) Knock-in of T2A-BirA* to the p53 C-terminus. The pAav-TP53-T2A-BirA* plasmid was packaged in rAAV and served as HDR repair template for the Cas9-induced DNA lesion site. After a first screening round to confirm integration, cells were treated with TAT-Cre for Cre-Lox recombination leading to the removal of the neomycin resistance cassette. The final HCT116 $TP53^{+/T2A\text{-}BirA^*}$ cell line contains only a remnant Lox site as a scar sequence. Cyan:

T2A sequence. Purple: CRISPR/Cas9. (B) PCR screening strategy. Arrows are indicative for primer location on the altered locus for PCR screening. Grey arrows: primers used for 5' HR PCR (1706 nt). Orange arrows: primers used for 3' HR PCR (1467 nt). Blue arrows: primers used for cassette deletion PCR (WT amplicon: 163 nt; recombined amplicon: 1230 nt; not recombined amplicon: 2879 nt). Asterisks indicate location of the primer outside the HR to exclude false-positive PCR amplicon in case of random integration.

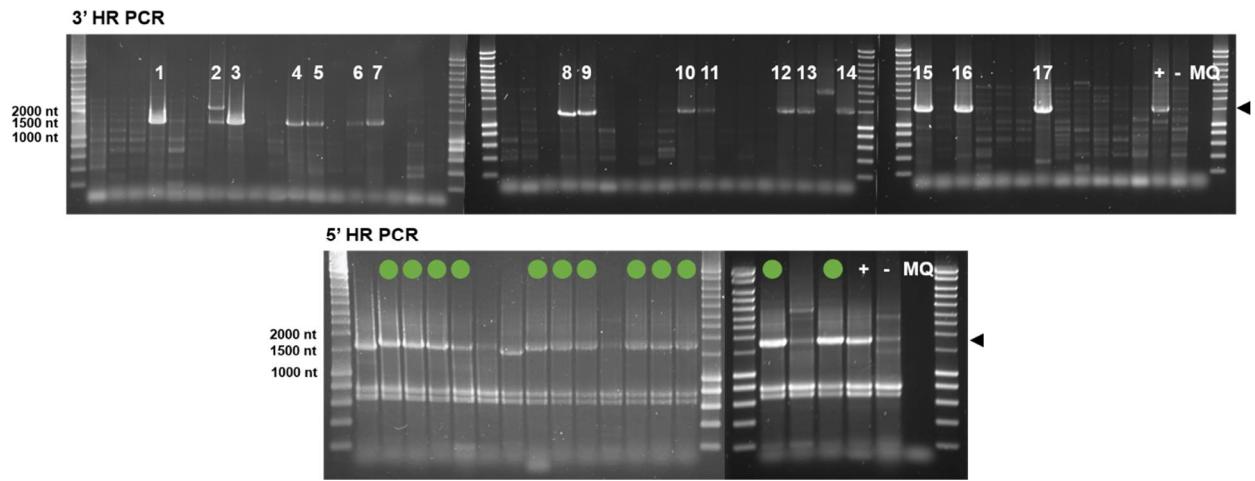


Figure S2. PCR screening of neomycin resistant clonal populations. PCR screening of clonal populations for HDR on agarose gels. Lysates were obtained by adding the cell suspension to DirectPCR lysis reagent. 17 clones showed a correct amplicon for 3' HR (1467 nt). These 17 clones were retained for the 5' HR PCR of which 12 were positive for the 5' HR PCR (1706 nt) as indicated in green. +: Positive control consisting of the neomycin resistant pool of HCT116 cells subjected to transfection and infection. -: Parental (non-engineered) HCT116 cells. MQ: No input DNA negative control.

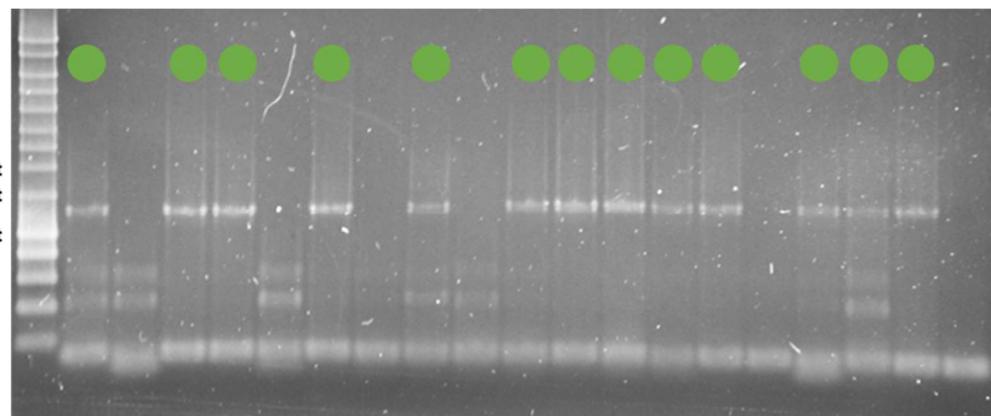


Figure S3. PCR for assessing the removal of the selection cassette upon TAT-Cre treatment. Agarose gel showing a typical post-recombination PCR screening. Lysates were obtained by adding the cell suspension to DirectPCR lysis reagent. Confirmed HDR positive clones were subjected to TAT-Cre treatment and screened by PCR for neomycin selection cassette removal by Cre-Lox recombination. WT amplicon: 163 nt; recombinant amplicon: 1230 nt; not recombinant amplicon: 2879 nt. 13 out of 19 clones showed recombination as indicated in green. Contrast adjusted to +40%. Empty lanes are possibly due to non-recombinant amplicons that were not amplified as elongation time did not allow for PCR amplification of this size. Alternatively, this can be due to varying amounts of input DNA obtained from the lysis reagent.

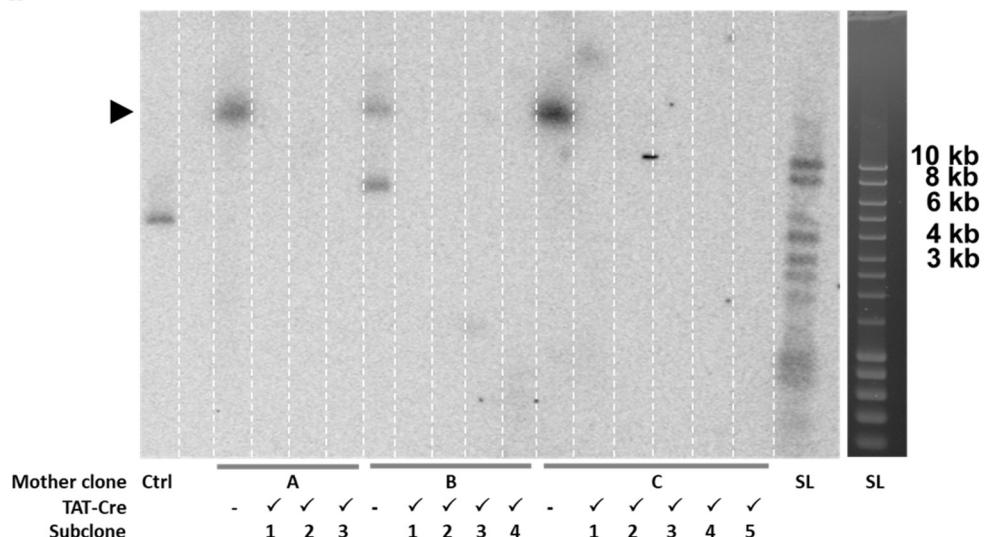
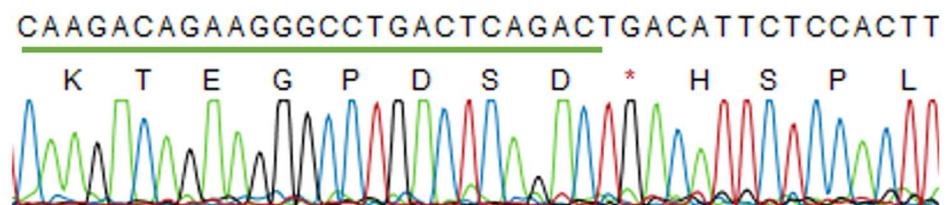
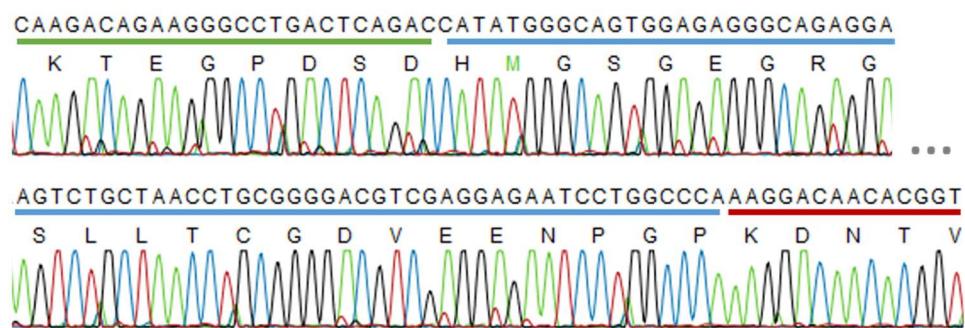
A.**B.****C.**

Figure S4. Validation of targeted T2A-BirA* insertion. (A) Southern blot of engineered T2A-BirA* clones prior and after TAT-Cre treatment to verify single integration and subsequent removal of the neomycin selection cassette. A total of 3, 4 and 5 TAT-Cre treated single cell subclones derived from 3 mother clones (A, B and C respectively) were assayed for cassette removal. A [α -³²P] Southern blot probe was generated against the neomycin resistance cassette. Expected size of the EcoRI digested HCT116 *TP53*^{+/-T2A-BirA*} DNA

fragment containing the probed selection cassette: \pm 20 kb. Ctrl: positive control from an ERN1 FLAG-tagged cell line containing the cassette with expected fragment size of 3537 bp. SL: Smartladder (left: post transfer on the membrane, right: SL on 0.7% agarose gel prior to transfer). Mother clone B showed double integration of the selection cassette, leading to exclusion of all derived post TAT-Cre expanded clones for further experiments. Clones 1-4 in the T2A mutagenesis Western blot experiment (Figure 2A) correspond to clone A1, A3, C1 and C3 respectively. (B) Sanger sequencing result of unaltered WT allele in knock-in clone C3 (clone 4). Green line: C-terminus of p53 (C) Sanger sequencing result of T2A-BirA* allele of the HCT116 $TP53^{+/T2A\text{-}BirA^*}$ clone C3 (clone 4). Blue line: Linker sequence and T2A sequence. Red line: N-terminus of BirA*

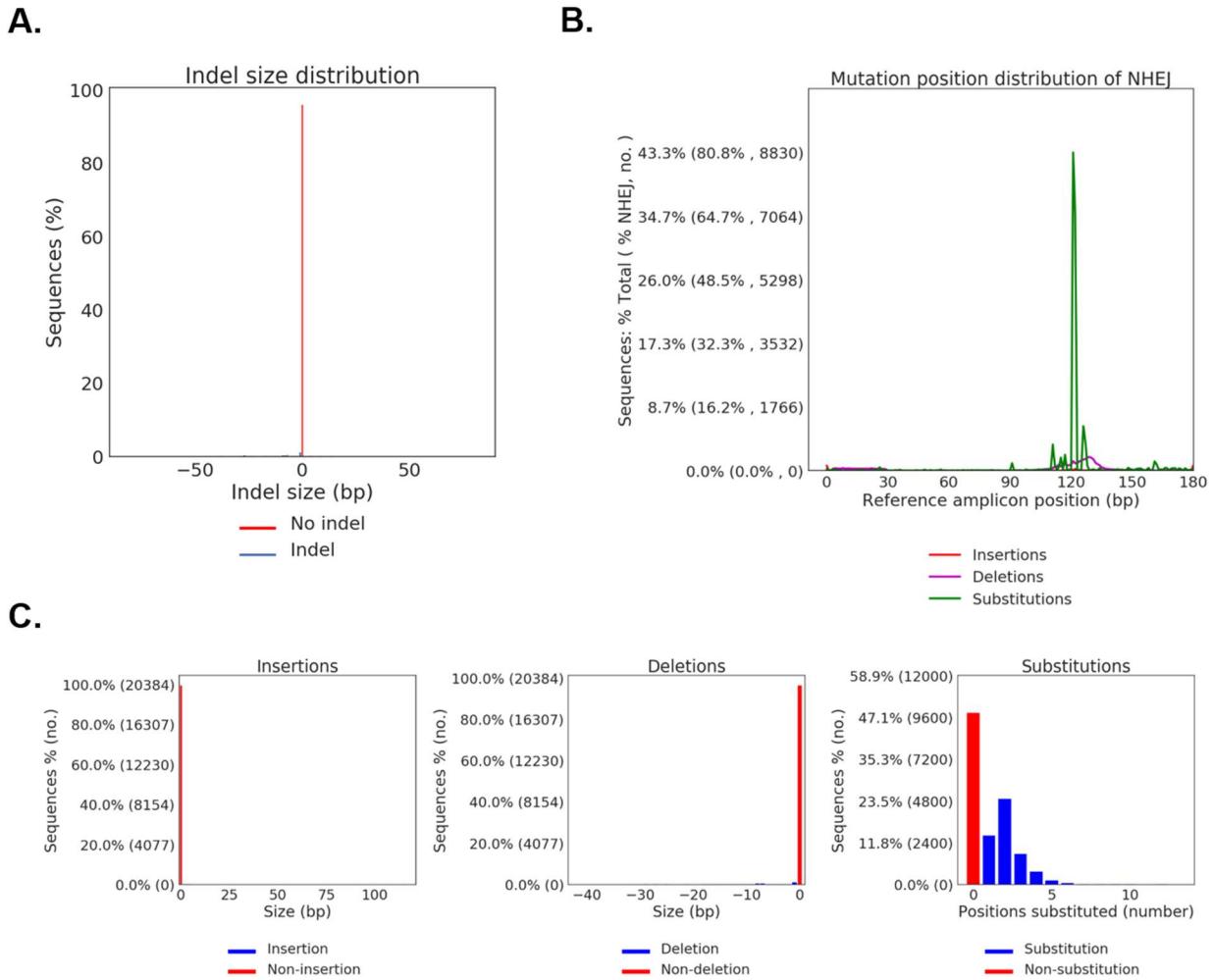


Figure S5. CRISPRESSO analysis of the T2A region after BE3 base editing. (A) Insertion and deletion (Indel) size distribution after BE3 treatment. Formation of indel mutations was negligible, with nearly all mutated amplicons bearing substitutions. (B) Position distribution of mutations in BE3-treated cells. Substitutions are exclusively prevalent in the mutation window of BE3. (C) Mutational size pattern of BE3-treated cells. Negligible insertions (left panel) and deletions (middle panel) were discerned. Substitutions (right panel) vary in size from 1 to 5 nucleotides, in accordance with the number of available residues in the T2A target sequence.

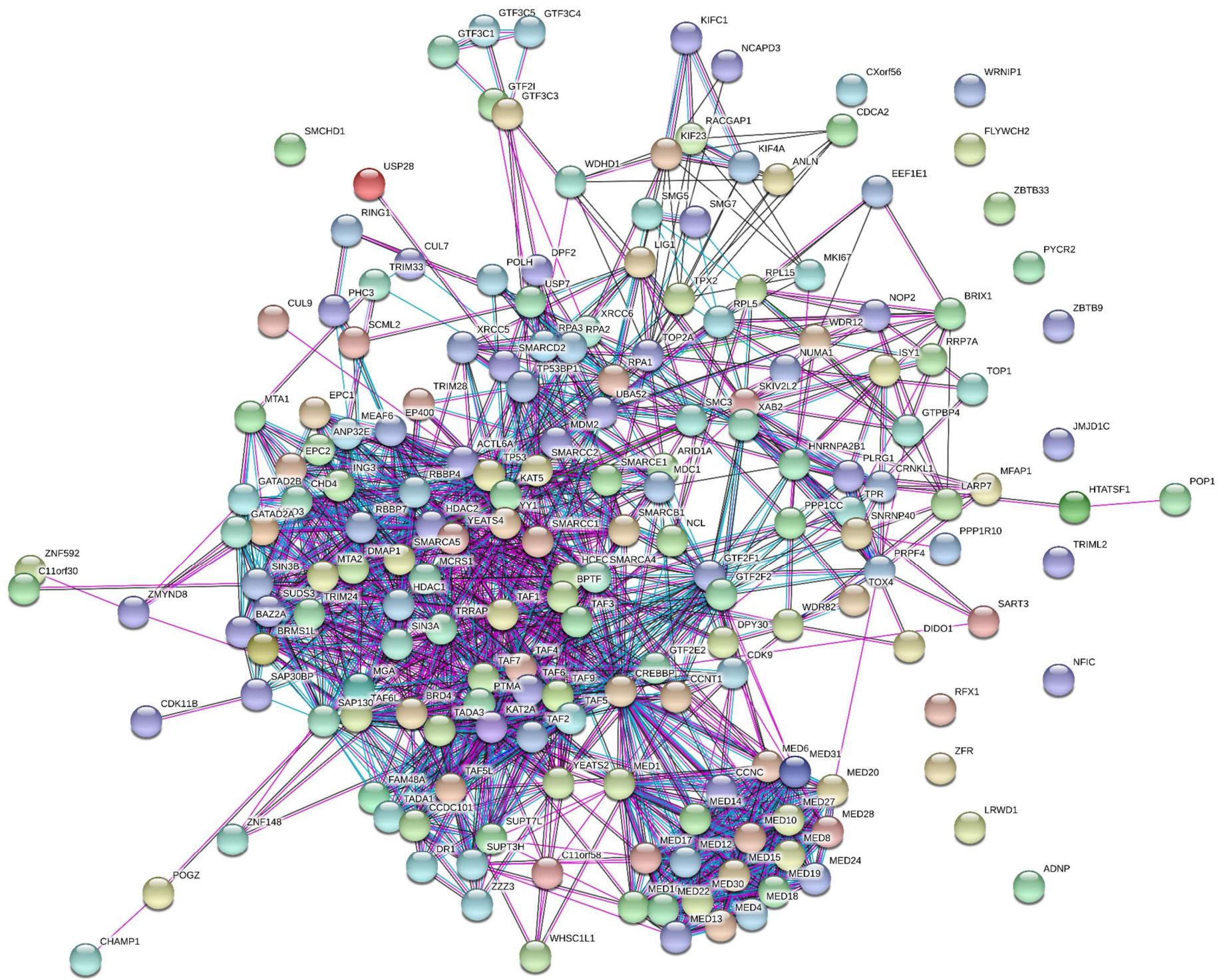


Figure S6. STRING analysis of proximal proteins for endogenous p53. All candidate proximal proteins detected in the edited p53-MUTT2A-BirA* HCT116 cells when isogenic p53-T2A-BirA* cells were used as control. Text mining associations are disabled. Minimum required interaction score was set on medium confidence (0.400).

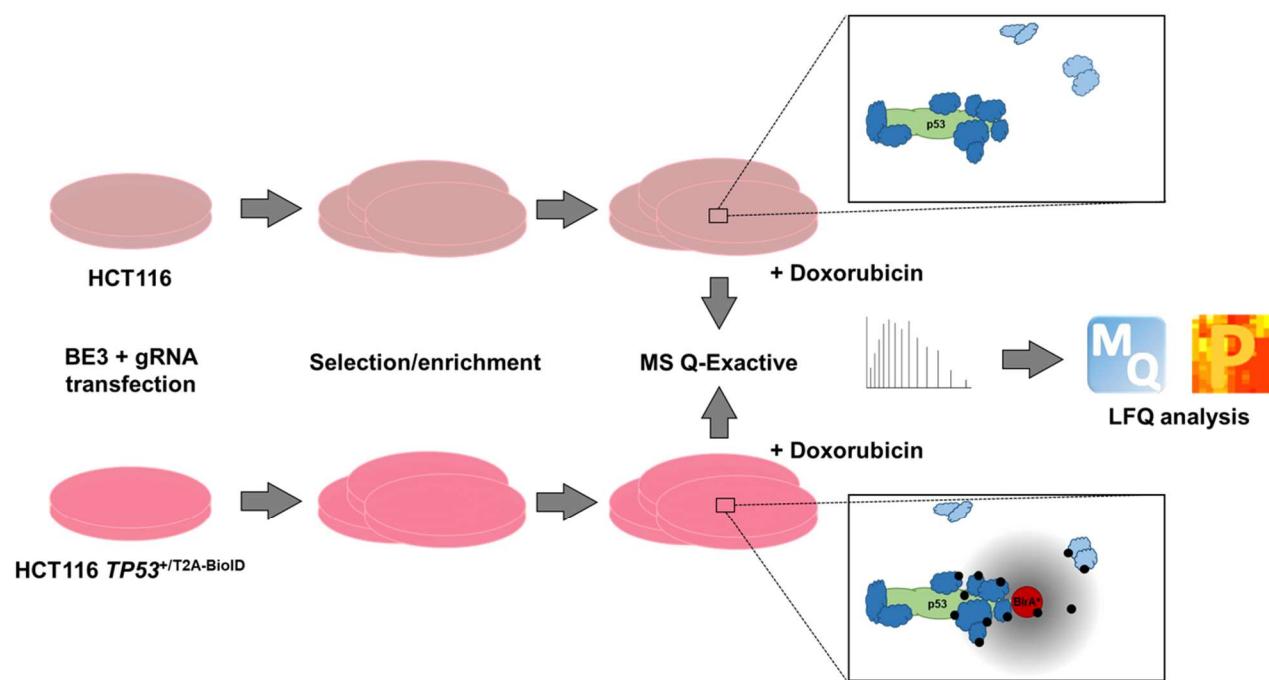
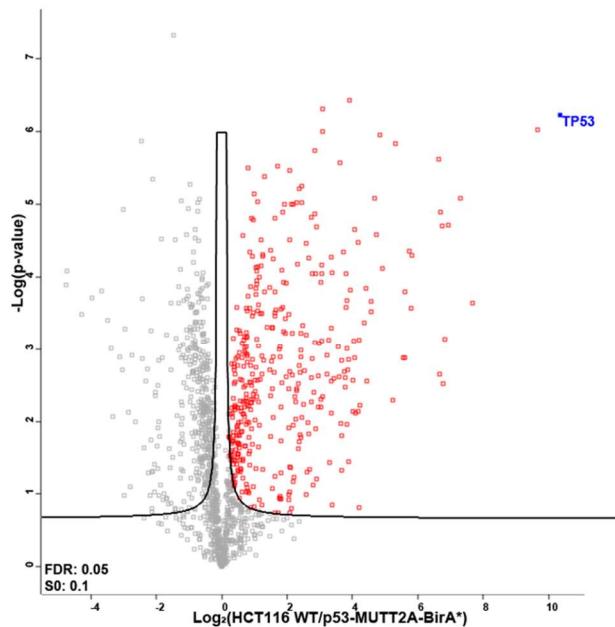
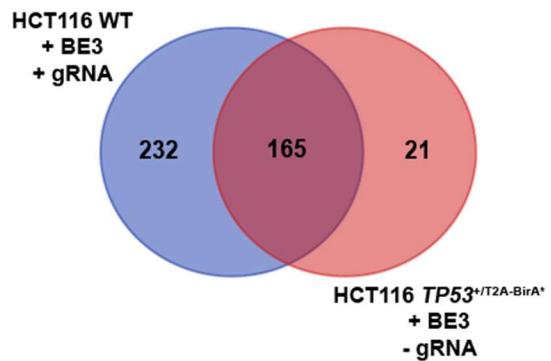
A.**B.****C.**

Figure S7. Evaluation of BE3 transfected non-engineered WT cells as control for p53 BioID. (A) Proteomics workflow when using transfected HCT116 WT cells as control condition. **(B)** Volcano plot showing the differential LFQ intensity levels (X-axis) and the p-values (Y-axis) for HCT116 *TP53*^{+/T2A-BirA*} after T2A conversion in presence of 1μM doxorubicin. **(C)** Venn diagram comparing significant hits in analyses using the two different control conditions. Comparing the two different controls showed an overlap of 165 significant proteins. 232 proteins were exclusively identified in a significant manner when using the HCT116 BE3-transfected control.